US Army Medical Research Institute of Chemical Defense

USAMRICD-TR-11-01

Evaluation of ADD392124 for the Delayed Treatment of Nerve Agent-Induced *Status Epilepticus* Seizures

John H. McDonough
Kerry E. Van Shura
Megan E. Lyman
Claire G. Eisner
Amelia Mazza
Robert K. Kan
Tsung-Ming Shih

September 2011

US Army Medical Research Institute of Chemical Defense
3100 Ricketts Point Road, Aberdeen Proving Ground, MD 21010-5400

an element of the

US Army Medical Research and Materiel Command

DISPOSITION INSTRUCTIONS:

Destroy this report when no longer needed. Do not return to the originator.

DISCLAIMERS:

The views expressed in this technical report are those of the author(s) and do not reflect the official policy of the Department of Army, Department of Defense, or the U.S. Government.

The experimental protocol was approved by the Animal Care and Use Committee at the United States Army Medical Research Institute of Chemical Defense, and all procedures were conducted in accordance with the principles stated in the Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966 (P.L. 89-544), as amended.

The use of trade names does not constitute an official endorsement or approval of the use of such commercial hardware or software. This document may not be cited for purposes of advertisement.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	**2. REPORT TYPE**	**3. DATES COVERED** *(From - To)*
September 2011	Technical	15 November 2009 - 30 January 2010

4. TITLE AND SUBTITLE

Evaluation of ADD392124 for the delayed treatment of nerve agent-induced *status epilepticus* seizures

5a. CONTRACT NUMBER

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)

McDonough, JH, Van Shura, KE, Lyman, ME, Eisner, CG, Mazza, A, Kan, RK, Shih, T-M

5d. PROJECT NUMBER

5e. TASK NUMBER

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)

US Army Medical Research Institute of Chemical Defense
ATTN: MCMR-CDR-P
3100 Ricketts Point Road
Aberdeen Proving Ground, MD 21010-5400

8. PERFORMING ORGANIZATION REPORT NUMBER

USAMRICD-TR-11-01

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)

US Army Medical Research Institute of Chemical Defense
ATTN: MCMR-CDZ-I
3100 Ricketts Point Road
Aberdeen Proving Ground, MD 21010-5400

10. SPONSOR/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)

12. DISTRIBUTION / AVAILABILITY STATEMENT

Approved for public release; distribution unlimited

13. SUPPLEMENTARY NOTES Supported by an Inter-Agency Agreement between NIH/NINDS (Y1-O6-9613-01) and USAMRICD (A120-B.P2009-2). This work was carried out under USAMRICD Research Protocol 1-08-U-915, "A rat model of nerve agent poisoning to evaluate delayed treatments with novel anticonvulsants," and the data are recorded in USAMRICD Notebook 006-09.

14. ABSTRACT

ADD392124 was identified by the Anticonvulsant Screening Program as being able to control benzodiazepine-resistant *status epilepticus* seizures. We evaluated the ability of ADD392124 to control seizures induced by the nerve agent soman. Rats were exposed to a convulsant dose of soman. Seizures were allowed to develop, and the standard treatment for nerve agent intoxication--atropine, 2-PAM (an oxime cholinesterase reactivator), and diazepam (a benzodiazepine)--was administered at either 5 or 20 min after seizures started along with ADD392124 at varying doses. ADD392124 was capable of stopping soman-induced seizures at both treatment delay times: anticonvulsant ED_{50} at the 5-min treatment delay = 256.1 mg/kg (226.3 – 336.9 mg/kg, 95% confidence limits); anticonvulsant ED_{50} for the 20-min treatment delay was 325.7 mg/kg (291.3 – 937.5 mg/kg, 95% confidence limits). The time for seizure termination following administration of ADD392124 at the 5-min treatment delay time was 533.9 sec (8.9 min), while the latency for seizure termination at the 20-min delay was 2258.3 sec (37.6 min). ADD392124 was less potent as an anticonvulsant when compared to anticholinergics, N-methyl-d-aspartate (NMDA) antagonists or benzodiazepines. Nevertheless, ADD392124 was successful in terminating soman-induced seizures at delay times (e.g., 20 min) where few other classes of anticonvulsant drugs have proven effective.

15. SUBJECT TERMS

Chemical warfare agents, nerve agents, seizures, anticonvulsants, therapy, delayed treatment, medical countermeasures

16. SECURITY CLASSIFICATION OF:			**17. LIMITATION OF ABSTRACT**	**18. NUMBER OF PAGES**	**19a. NAME OF RESPONSIBLE PERSON**
a. REPORT	**b. ABSTRACT**	**c. THIS PAGE**			John H. McDonough
UNCLASSIFIED	UNCLASSIFIED	UNCLASSIFIED	UNLIMITED	16	**19b. TELEPHONE NUMBER** *(include area code)* 410-436-1942

Standard Form 298 (Rev. 8-98)
Prescribed by ANSI Std. Z39.18

Abstract

ADD392124 was identified by the Anticonvulsant Screening Program as being able to control benzodiazepine-resistant lithium-pilocarpine-induced *status epilepticus* seizures, a seizure model that closely resembles nerve agent-induced seizures. The present research evaluated the ability of ADD392124, delivered as an adjunct to standard antidotes, to control seizures induced by the nerve agent soman when treatment was delayed for 5 or 20 min after seizure onset. Rats, previously prepared with electrodes to record electroencephalographic (EEG) activity, were exposed to a convulsant dose of the nerve agent soman. Seizures were allowed to develop, and the standard treatment for nerve agent intoxication—atropine, 2-PAM (an oxime cholinesterase reactivator), and diazepam (a benzodiazepine)—was administered at either 5 or 20 min after seizures started. This standard treatment alone was unable to stop seizure activity. ADD392124 was administered along with these standard treatments at various doses to determine effective doses for termination of these seizures. ADD392124 was capable of stopping soman-induced seizures at both treatment delay times. The ED_{50} for seizure control at the 5-min treatment delay was calculated by probit analysis to be 256.1 mg/kg (226.3 – 336.9 mg/kg, 95% confidence limits), while the ED_{50} dose for the 20-min treatment delay was 325.7 mg/kg (291.3 – 937.5 mg/kg, 95% confidence limits). The time for seizure termination following administration of ADD392124 at the 5-min treatment delay time was 533.9 sec (8.9 min), while the latency for seizure termination at the 20-min delay was 2258.3 sec (37.6 min). As with other anticonvulsant treatments that have been studied in this model, animals in which the seizures were terminated by ADD392124 displayed minimal or no neuropathology, while animals in which the treatment was unsuccessful in stopping the seizures displayed significant levels of neuropathology in those brain areas susceptible to nerve agent-induced damage. In comparison to other drugs that have been studied using this model, ADD392124 was less potent as an anticonvulsant when compared to anticholinergics, N-methyl-d-aspartate (NMDA) antagonists and drugs that enhance gamma-amino butyric acid (GABA) receptor activity. Nevertheless, ADD392124 was successful in terminating soman-induced seizures at delay times (e.g., 20 min) where few other classes of anticonvulsant drugs have proven effective.

Background

Nerve agents are organophosphorous compounds that irreversibly inhibit the enzyme acetylcholinesterase. This leads to a rapid accumulation of the neurotransmitter acetylcholine and an overstimulation of cholinergic effector sites in the brain and periphery. A notable sign of nerve agent poisoning is the development of prolonged *status epilepticus* seizures (McDonough et al., 1995; McDonough and Shih, 1997). Nerve agent-induced seizures result from overstimulation of susceptible brain circuits by abnormally high levels of the excitatory neurotransmitter acetylcholine that rapidly builds up after inhibition of the enzyme acetylcholinesterase by nerve agent (McDonough and Shih, 1997). These seizures, unless quickly stopped pharmacologically, rapidly progress to *status epilepticus*, a state of continuous seizure activity or episodes of seizure activity for greater than 30 min with no recovery of consciousness between episodes. *Status epilepticus* itself is considered a medical emergency, and the longer seizures persist the more difficult they are to stop pharmacologically (Shorvon, 1994).

Status epilepticus clinically responds (n.b., termination of seizures) only to a subset of anticonvulsant or antiepileptic drugs. Benzodiazepines are typically the most effective class of compounds and are used as the drug of first choice in treatment of this condition clinically. Benzodiazepines, barbiturates and compounds with N-methyl-d-aspartate (NMDA) antagonist activity (e.g., ketamine, MK-801), as well as potent anticholinergic compounds, are the only compounds that have been shown to be effective against nerve agent-induced *status epilepticus* seizures. In addition, like *status epilepticus* seizures elicited by other means, nerve agent-induced seizures become refractory to treatment with benzodiazepines and anticholinergic drugs the longer treatment is delayed (McDonough and Shih, 1993, 1997; McDonough et al., 2010; Shih et al., 2003, 2007).

The present series of experiments were performed to evaluate the anticonvulsant potential of the drug ADD392124. This drug was identified as being effective in terminating benzodiazepine-resistant *status epilepticus* seizures elicited by lithium-pilocarpine in a rat model utilized by the Anticonvulsant Screening Program (ASP) run at The University of Utah and sponsored by the NINDS. Seizures elicited in the lithium-pilocarpine model have many electroencephalographic (EEG) and pharmacological characteristics that are similar to nerve agent-induced seizures. Following the positive tests in the ASP, a material transfer agreement (MTA) was prepared between the developer of ADD392124 and the US Army Medical Research Institute of Chemical Defense (USAMRICD). This allowed the transfer of a sample of ADD392124 to USAMRICD for testing in a rat model of nerve agent-induced seizures that was developed with the support of an Inter-Agency Agreement between NIH/NINDS and USAMRICD. This rat model was initially developed in the late 1980s (Capacio and Shih, 1991; Shih et al., 1991), and variants of this model have been used to test the effectiveness of anticonvulsant compounds from a variety of pharmacological classes for the treatment of nerve agent-induced seizures (McDonough and Shih, 1993; Shih et al., 1999).

Methods

Subjects: Male Sprague-Dawley rats (Crl:DCBR VAF/Plus) from Charles River Labs, weighing 250-300 g upon receipt, served as subjects. The animals were housed individually in temperature (21$\pm$ 2^0 C) and humidity (50 $\pm$ 10%) controlled quarters and maintained on a 12-h light-dark full spectrum lighting cycle with lights on at 0600. Laboratory rat chow and tap water were freely available.

Surgery: Each animal was anesthetized with isoflurane (5% induction; 3-1.5% maintenance, with oxygen) and placed in a stereotaxic instrument. Two stainless steel screws were placed in the skull bilaterally midway between bregma and lamda and ~3 mm lateral to the midline. A third screw was placed over the cerebellum. The screws were connected to a miniature connector with wires and the screws, wires and connector were then anchored to the skull with dental cement. The incision was sutured; the animal was removed from the frame, given the analgesic buprenorphine HCl (0.03 mg/kg, SC) and placed on a warming pad for at least 30 min before being returned to the animal quarters. Approximately seven days elapsed between surgery and experimentation.

Materials: Saline (0.9% NaCl) injection, USP, was purchased from Cutter Labs, Inc. (Berkeley, CA). The oxime HI-6 DiCl (1-(((4-(aminocarbonyl)pyridinio) methoxy)methyl)-2-((hydroxyimino)methyl)pyridinium dichloride) was obtained from the depository at the Division of Experimental Therapeutics, Walter Reed Army Institute of Research (Silver Spring, MD). The oxime pyridine-2-aldoxime methylchloride (2-PAM) was purchased from Ayerst Labs, Inc. (New York, NY). Atropine sulfate and atropine methyl nitrate were purchased from Sigma-Aldrich Chemical Company (St. Louis, MO). Attane^{TM} (isoflurane, USP) was purchased from Minrad, Inc. (Bethlehem, PA). Buprenorphine HCl was purchased from Reckitt Benckiser Pharmaceuticals, Inc. (Richmond, VA). Diazepam was purchased from T.W. Medical Co. (Lago Vista, TX). The nerve agent soman was obtained from the US Army Edgewood Chemical Biological Center (Aberdeen Proving Ground, MD). HI-6 (250 mg/ml), atropine methyl nitrate (4.0 mg/ml), atropine sulfate (0.2 mg/ml) admixed with 2-PAM (50.0 mg/ml) and soman (360 µg/ml) were prepared in saline, either fresh daily (HI-6, atropine methyl nitrate, atropine sulfate + 2-PAM) or as frozen aliquots (soman). ADD392124 was received and maintained at room temperature in a dessicator until use. ADD392124 was prepared in 0.5% methyl cellulose fresh each experimental day at concentrations to deliver volumes = 2.0 ml/kg. Because of the poor solubility of ADD392124 in aqueous solutions, limited studies used DMSO and a DMSO (15%) + polyethylene glycol (PEG; 75%) mixture as vehicles.

Anticonvulsant Test: Animals were typically tested in squads of eight on a given study day. The animals were randomized among treatment groups each test day. The animals were weighed, placed in individual recording chambers and connected to the recording apparatus. EEG signals were recorded using CDE 1902 amplifiers and displayed on a computer running Spike2 software (Cambridge Electronic Design, Ltd., Cambridge, UK). Baseline EEG was recorded for at least

20 min. The animals were then pretreated with 125 mg/kg, IP, of the oxime HI-6 to prevent the rapid lethal effects of the soman challenge. Thirty min later the animals were challenged with 180 μg/kg, SC, soman (1.6 X LD_{50}) and 1 min later treated with 2.0 mg/kg, IM, atropine methyl nitrate to minimize peripheral secretions. The animals were then closely monitored both visually and on the EEG for seizure onset. Seizure onset was operationally defined as the appearance of >10 sec of rhythmic high amplitude spikes or sharp waves that were at least twice the baseline amplitude accompanied by a rhythmic bilateral flicking of the ears, facial clonus and possibly forepaw clonus. At 5 or 20 min after seizure onset, the animals received standard medical countermeasures: 0.1 mg/kg atropine sulfate + 25 mg/kg 2-PAM Cl admixed to deliver 0.5 ml/kg, IM, and 0.4 mg/kg, IM, diazepam. These standard medical countermeasures are not sufficient, by themselves, to terminate soman-induced seizures. Immediately after administering the standard medical countermeasures, individual animals received an IP dose of ADD392124 (see Table 1 for doses used). The animals were monitored on the EEG for at least 5 hr after exposure and then returned to the animal housing room. Twenty-four hr after the exposure, the surviving animals were weighed and the EEG again recorded for at least 30 min. Following this, the animals were administered an anesthetic dose (75 mg/kg, IP) of pentobarbital and when deeply anesthetized were perfused intracardially with saline followed by formalin. The brain was harvested, blocked in the coronal plane at the level of the infindibulum and then embedded in paraffin. This insures that nearly identical brain areas are examined from animal to animal. Sections were cut 6-10 μm thick and stained with hematoxylin and eosin. Eight brain areas (dorsal and lateral cerebral cortex, pyriform cortex, amygdala, hippocampus – CA1, CA4/dentate hillus, CA2/CA3, thalamus) were evaluated in each animal using a 0-4 rating scale (0 = No lesion; 1 = Minimal [1-10%]; 2 = Mild [11-25%]; 3 = Moderate [26-45%]; 4 = Severe [>45%] based on the percentage tissue involvement) and then summed to obtain a total neuropathology score. Evaluation and categorization of the EEG response by an individual animal to treatment were performed by a technician and investigator, both well-experienced with the appearance of nerve agent-induced EEG seizure activity. The overall rating and timing of different events required consensus between both individuals, who were aware of the treatment conditions of an individual animal. To be rated as having the seizure terminated, all spiking and/or rhythmic waves had to stop and the EEG had to remain normal at all subsequent observation times (n.b., throughout the 5-hr record following exposure and for the 30-min record 24 hr later). For each animal in which the seizure was terminated, the latency to seizure termination was measured as the time from when the animal received the ADD392124 treatment to the last observable epileptiform event in the EEG.

Data Analysis: The median effective dose for anticonvulsant activity was determined by probit analysis (Bliss, 1952). Seizure termination latencies were calculated from the time ADD392124 was administered to the termination of EEG spiking and/or rhythmic waves and were evaluated using t-tests; incidence and severity of neuropathology were evaluated with Fisher's exact test and a one-way ANOVA, respectively. In all cases, $p<0.05$ was considered significant.

Results

Table 1 displays the doses used, the numbers of animals tested at each treatment delay time, the fractional response and the percentage of animals displaying an anticonvulsant response.

Table 1. Experimental summary of ADD392124 doses tested and the anticonvulsant response of animals at each treatment delay.

Test Dose ADD392124	5-min Treatment Delay	20-min Treatment Delay
140 mg/kg	0/6, 0%	NT
180 mg/kg	0/8, 0%	0/5, 0%
225 mg/kg	4/9, 44%	0/6, 0%
280 mg/kg	4/7, 57%	3/6, 50%
320 mg/kg	NT	6/15, 40%

NT = not tested
Number of animals responding (anticonvulsant)/number of animals tested

At the 5-min treatment delay time, ADD392124 produced a significant anticonvulsant effect with an ED_{50} = 256 mg/kg (226-337 mg/kg = 95% confidence limits). The idealized dose-effect curve for these data is displayed in Figure 1, and a copy of the probit analysis output is included as Appendix 1. There were five animals, 1 at 140 mg/kg, 2 at 180 mg/kg and 2 at 225 mg/kg ADD392124, that had an initial anticonvulsant response to the treatment and then the seizures returned at a later time; these animals were considered treatment failures. There were also two animals at the 280 mg/kg dose that had their seizures terminated, but failed to survive 24 hr; these animals were considered treatment successes. The average latency for seizure termination at the 5-min treatment delay time was 534 sec (8.9 min) (196 – 871 sec = 95% confidence limits), and this is displayed graphically in Figure 2.

At the 20-min treatment delay time, ADD392124 failed to produce a statistically significant anticonvulsant effect due to the low number of responders in the 320 mg/kg treatment group. Because of this, while an anticonvulsant ED_{50} could be calculated by probit analysis (20-min delay anticonvulsant ED_{50} = 321 mg/kg ADD392124), no confidence limits could be determined. The idealized dose-effect curve for these data is displayed in Figure 1, and a copy of the probit analysis output is included as Appendix 2. As mentioned in the methods, DMSO and DMSO + PEG were also used as solvents in a limited number of animals (DMSO = 5; DMSO + PEG = 4) at the 320 mg/kg dose. These solvents produced about the same response rate as when ADD392124 was used in 0.5% methyl cellulose. Specifically, a 2 of 5 response rate for DMSO and a 2 of 4 response rate for DMSO + PEG. When all the solvents for the 320 mg/kg dose of ADD392124 were factored into a probit analysis, it resulted in a significant result with an ED_{50} = 326 mg/kg (291 – 938 mg/kg = 95% confidence limits), a dose comparable to that obtained with just the methyl cellulose vehicle animals alone. The dose-effect curve for this result is presented in Figure 3. The copy of this probit analysis output is included as Appendix 3. As at the 5 min

test, there were five animals, 3 at 225 mg/kg and 2 at 320 mg/kg, that had an initial anticonvulsant response to the treatment and then the seizures returned at a later time; these animals were considered treatment failures. There were also two animals, one each at the 180 and the 280 mg/kg doses that had their seizures terminated, but failed to survive 24 hr; these animals were considered treatment successes. The average latency for seizure termination at the 20-min treatment delay time was 2258 sec (37.6 min) (1091 - 3426 sec = 95% confidence limits) and this is displayed graphically in Figure 2. The latency for seizure control at the 20-min treatment delay time was significantly ($t = 3.52$, $df = 39$, $p < 0.01$) longer than the comparable latency at the 5-min treatment delay time.

Brains were examined microscopically for evidence of neuropathology. As observed in other studies, evidence of neuropathology was strongly related to the ability of the test drug to control seizure activity. Table 2 displays the numbers of animals that either did or did not display neuropathology when animals were classified as Treatment Success vs Treatment Failure. As can be seen from the table, animals classified as Treatment Failures overwhelmingly displayed neuropathology when compared to animals classified as Treatment Successes. A Fisher's exact test confirmed this impression, and showed a highly significant ($p < 0.001$) difference between groups. Of the seven animals in the Treatment Success/Neuropathology classification, six of them were treated at the 20 min treatment delay time. Animals in the Treatment Success/ Neuropathology classification had significantly ($t = 4.50$, $df = 46$, $p < 0.001$) lower total neuropathology scores ($X = 1.7$) when compared to the total neuropathology scores of the animals in the Treatment Failure/Neuropathology classification ($X = 12.10$). These data all show that successful anticonvulsant action by ADD392124 either totally prevented or significantly minimized the brain pathology in surviving animals.

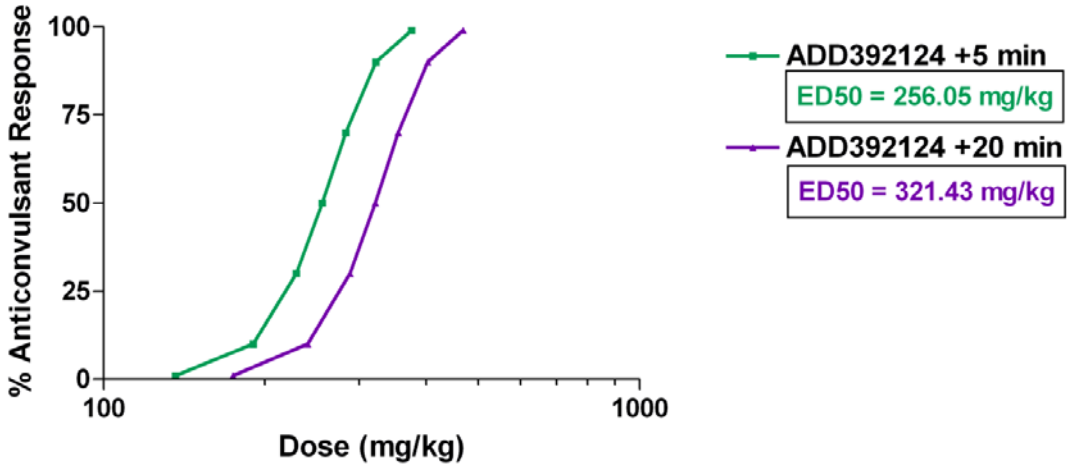

Figure 1**.** Idealized dose-effect curves for ADD392124 when administered at the 5-min or 20-min treatment delay times. These curves contain data only from methyl cellulose vehicle treated animals.

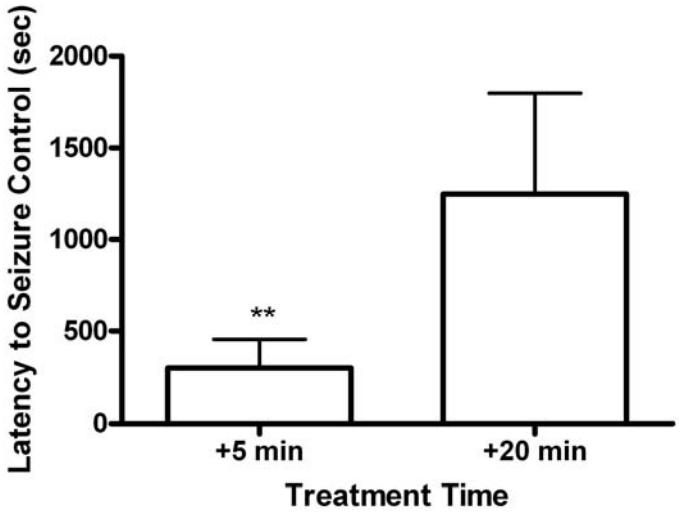

Figure 2. Seizure control latencies following successful anticonvulsant treatment with ADD392124 at either the 5-min or 20-min treatment delay times. **Significantly ($p < 0.01$) shorter seizure control latency at the 5-min compared to 20-min treatment delay time.

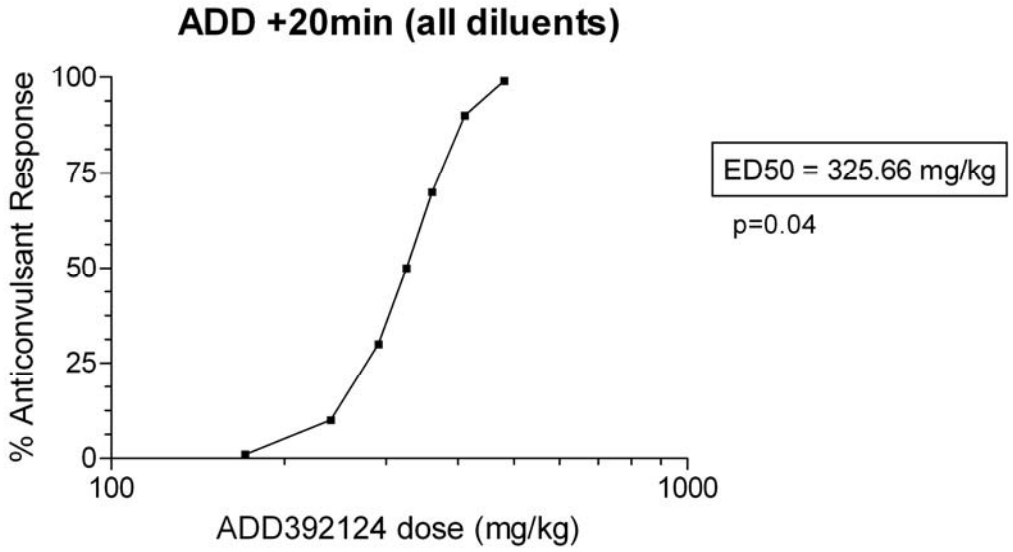

Figure 3. Idealized dose-effect curve for ADD392124 administered at the 20-min treatment delay time. This curve contains data from animals treated with all vehicles at the 320 mg/kg dose.

Table 2. Numbers of animals with or without neuropathology and associated mean neuropathology score ( ) as categorized by anticonvulsant response to ADD392124.

	Neuropathology	No Neuropathology
Seizure Off – Treatment Success	7 (X = 1.71)	6
Seizure Not Off – Treatment Failure	42 (X = 12.1)	1

Note: data collapsed across both treatment delay times.
Both the incidence (Fisher's exact test, $p < 0.01$) and severity of neuropathology (t test, $p < 0.01$) were significantly influenced by treatment success or failure.

Discussion

ADD392124 was effective in terminating *status epilepticus* seizures elicited by the nerve agent soman in rats when treatment was delivered either at 5 or 20 min following seizure onset. As with other compounds tested with this animal model, higher drug doses were required to terminate seizures and it took the seizures longer to stop at the 20-min treatment delay than at the 5- min treatment delay. Successful anticonvulsant treatment was also associated with the prevention or significant reduction of brain pathology produced by the seizures.

The ED_{50}s for this anticonvulsant effect were 256 and 326 mg/kg for the 5- and the 20-min treatment delay times, respectively. The fact that a higher dose of ADD392124 was required at the 20-min treatment delay time is a common finding with this animal model with either anticholinergic drugs or benzodiazepines (McDonough and Shih, 1993; Shih et al., 1999). However, it is notable that the shift in ED_{50}s for these other compounds (anticholinergics or benzodiazepines) is of a substantially greater magnitude, on the order of 0.5 – 1.0 log unit, while the shift in the curves for ADD392124 was only ~0.1 log unit. Thus, while ADD392124 may be substantially less potent on a mg basis when compared to other compounds that can control nerve agent seizures, it does differ in the fact that it retains potency at longer treatment delays.

The time for seizure control seen at the 5-min treatment delay time, 8.9 min, is comparable to the times it takes anticholinergics or benzodiazepines to control soman-induced seizures under these conditions (McDonough and Shih, 1993; Shih et al., 1999). Also, the increase in the latencies for seizure control with longer treatment delays (e.g., 20 min) is commonly observed with other drugs in this model, so the fact that this was observed with ADD392124 should not be considered unusual.

Control of soman-induced seizures with ADD392124 successfully protected animals against the severe neuropathology that is typically observed in the brains of animals exposed to nerve agents (Carpentier et al., 1990; McDonough et al., 1995). Some of the successfully treated 20-min treatment delay animals developed minimal amounts of neuropathology. This has been observed in other experiments, and it has been concluded that as little as 20 min of intense

seizure activity will already have set up a pathological process in vulnerable neurons that will be observed histopathologically when the animal is euthanized 24 hr later (Lallement et al., 1994; McDonough et al., 1995). The fact that the pathology scores of this treatment success group were significantly lower than the animals in which seizures were not stopped shows how important control of seizure activity is to this process.

ADD392124 proved difficult to put into solution. While it suspended well in 0.5% methyl cellulose, injection IP into an animal using anything <18 gage needle proved problematic. Other solvents were tested, but this was limited due to the small amount of test compound available. ADD392124 did dissolve in DMSO and also could be dissolved in limited amounts of DMSO and then diluted to a final concentration with PEG. Both of these formulations appeared to have the same potency as when 0.5% methyl cellulose was used. Formulations of ADD392124 or analogs of this compound that dissolved in more aqueous solutions may have a more potent and rapid anticonvulsant effect.

In summary, ADD392124 provided effective anticonvulsant action against seizures induced in rats by the nerve agent soman when it was administered at 5 or 20 min after seizure onset. As with other compounds tested with this animal model, higher drug doses were required to terminate seizures, and it took the seizures longer to stop at the 20-min treatment delay than at the 5-min treatment delay. Successful anticonvulsant action with ADD392124 was able to prevent or minimize seizure-induced brain pathology, another result that is commonly seen in this model. It is speculated that improved formulations of ADD392124 may enhance the anticonvulsant effect.

References

Bliss, C.I. The statistics of bioassay with special reference to the vitamins. In: Vitamin Methods, Vol. II. Academic Press: New York, 1952.

Capacio, B.R., Shih, T.-M. Anticonvulsant actions of anticholinergic drugs in soman poisoning. Epilepsia, 1991, 32:604-615.

Carpentier, P., Delamanche, I.S., LeBert, M., Blanchet, G. Bouchaud, C. Seizure-related opening of the blood-brain barrier induced by soman: possible correlation with the acute neuropathology observed in poisoned rats. Neurotoxicology, 1990, 11:493-508.

Lallement, G., Pernot-Marino, I., Baubichon, D., Burkhart, M.-F., Carpentier, P., Blanchet, G. Modulation of soman-induced neuropathology with an anticonvulsant regimen. NeuroReport, 1994, 5:2265-2268.

McDonough, J.H. and Shih, T.-M. Pharmacological modulation of soman-induced seizures. Neuroscience and Biobehavioral Reviews, 1993, 17:203-215.

McDonough, J.H., Dochterman, L.W., Smith, C.D. and Shih, T.-M. Protection against nerve agent-induced neuropathology, but not cardiac pathology, is associated with the anticonvulsant action of drug treatment. Neurotoxicology, 1995, 15:123-132.

McDonough, J.H., McMonagle, J.D., Shih, T.-M. Time-dependent reduction in the anticonvulsant effectiveness of diazepam against soman-induced seizures in guinea pigs. Drug and Chemical Toxicology, 2010, 33(3):279-283.

McDonough, J.H. and Shih, T.-M. Neuropharmacological mechanisms of nerve agent-induced seizures and neuropathology. Neuroscience and Biobehavioral Reviews, 1997, 21:559-579.

Shih, T.-M., McDonough, J.H. and Koplovitz, I. Anticonvulsants for soman-induced seizure activity. Journal of Biomedical Science, 1999, 6:86-96.

Shih, T.-M, Duniho, S.M., and McDonough, J.H. Control of nerve agent-induced seizures is critical for neuroprotection and survival. Toxicology and Applied Pharmacology, 2003, 188:69-80.

Shih, T.-M., Koviak, T.A., Capacio, B.R. Anticonvulsants of poisoning by the organophosphorus compound soman: pharmacological mechanisms. Neuroscience and Biobehavioral Reviews, 1991, 15:349-362.

Shih, T.-M., Rowland, T.C., McDonough, J.H. Anticonvulsants for nerve agent-induced seizures: the influence of the therapeutic dose of atropine. Journal of Pharmacology and Experimental Therapeutics, 2007, 320:154-161.

Shorvon, S. Status epilepticus: its clinical features and treatment in children and adults. Cambridge University Press, Cambridge, UK, 1994, 244-254.

Appendix 1: 5-min treatment delay probit analysis

		95% Confidence Limits for dose		
	Probability	Estimate	Lower Bound	Upper Bound
PROBIT	.010	136.281	-143.818	183.692
	.020	150.316	-91.494	192.694
	.030	159.220	-58.460	198.569
	.040	165.919	-33.726	203.105
	.050	171.368	-13.701	206.890
	.060	176.005	3.259	210.194
	.070	180.072	18.054	213.168
	.080	183.713	31.229	215.903
	.090	187.024	43.141	218.460
	.100	190.072	54.037	220.883
	.150	202.692	98.171	231.893
	.200	212.722	131.445	242.445
	.250	221.327	157.758	253.732
	.300	229.054	178.682	266.572
	.350	236.215	195.111	281.432
	.400	243.009	207.913	298.320
	.450	249.583	218.026	316.932
	.500	256.053	226.298	336.930
	.550	262.523	233.373	358.125
	.600	269.096	239.705	380.518
	.650	275.891	245.620	404.293
	.700	283.052	251.369	429.832
	.750	290.779	257.184	457.783
	.800	299.384	263.324	489.242
	.850	309.414	270.172	526.221
	.900	322.034	278.465	573.072
	.910	325.082	280.428	584.428
	.920	328.393	282.546	596.779
	.930	332.034	284.859	610.375
	.940	336.100	287.425	625.577
	.950	340.738	290.332	642.936
	.960	346.187	293.723	663.353
	.970	352.886	297.861	688.485
	.980	361.790	303.319	721.937
	.990	375.825	311.838	774.744

Parameter Estimates

						95% Confidence Interval	
	Parameter	Estimate	Std. Error	Z	Sig.	Lower Bound	Upper Bound
PROBIT[a]	dose	.019	.007	2.662	.008	.005	.034
	Intercept	-4.973	1.735	-2.866	.004	-6.709	-3.238

Appendix 2. 20-min delay probit analysis; methyl cellulose data only

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	dose	.016	.009	1.849	.064	-.001	.033
	Intercept	-5.087	2.458	-2.070	.038	-7.544	-2.629

a. PROBIT model: PROBIT(p) = Intercept + BX

Confidence Limits

	Probability	95% Confidence Limits for dose		
		Estimate	Lower Bound	Upper Bound
PROBIT	.010	174.423	.	.
	.020	191.649	.	.
	.030	202.578	.	.
	.040	210.799	.	.
	.050	217.487	.	.
	.060	223.179	.	.
	.070	228.170	.	.
	.080	232.639	.	.
	.090	236.703	.	.
	.100	240.444	.	.
	.150	255.933	.	.
	.200	268.243	.	.
	.250	278.804	.	.
	.300	288.288	.	.
	.350	297.077	.	.
	.400	305.416	.	.
	.450	313.485	.	.
	.500	321.425	.	.
	.550	329.366	.	.
	.600	337.434	.	.
	.650	345.773	.	.
	.700	354.562	.	.
	.750	364.046	.	.
	.800	374.607	.	.
	.850	386.917	.	.
	.900	402.406	.	.
	.910	406.147	.	.
	.920	410.212	.	.
	.930	414.680	.	.
	.940	419.671	.	.
	.950	425.363	.	.
	.960	432.051	.	.
	.970	440.272	.	.
	.980	451.201	.	.
	.990	468.427	.	.

Appendix 3. 20-min delay probit analysis; all solvents included

Parameter Estimates

	Parameter	Estimate	Std. Error	Z	Sig.	95% Confidence Interval	
						Lower Bound	Upper Bound
PROBIT[a]	dose	.015	.007	2.053	.040	.001	.029
	Intercept	-4.892	2.194	-2.229	.026	-7.087	-2.698

a. PROBIT model: PROBIT(p) = Intercept + BX

Confidence Limits

	Probability	95% Confidence Limits for dose		
		Estimate	Lower Bound	Upper Bound
PROBIT	.010	170.812	-2520.524	237.511
	.020	188.957	-2119.071	247.574
	.030	200.470	-1864.511	254.108
	.040	209.130	-1673.126	259.134
	.050	216.175	-1517.541	263.313
	.060	222.171	-1385.197	266.954
	.070	227.428	-1269.237	270.226
	.080	232.136	-1165.486	273.234
	.090	236.417	-1071.207	276.047
	.100	240.358	-984.503	278.717
	.150	256.674	-626.814	291.058
	.200	269.641	-345.613	303.946
	.250	280.766	-110.795	321.431
	.300	290.757	82.022	355.188
	.350	300.015	207.158	440.008
	.400	308.800	257.976	588.418
	.450	317.299	279.120	760.028
	.500	325.663	291.325	937.521
	.550	334.028	300.161	1118.385
	.600	342.527	307.481	1303.819
	.650	351.312	314.090	1496.438
	.700	360.570	320.430	1700.053
	.750	370.560	326.822	1920.236
	.800	381.685	333.586	2165.774
	.850	394.653	341.162	2452.286
	.900	410.969	350.391	2813.089
	.910	414.910	352.583	2900.270
	.920	419.191	354.952	2994.994
	.930	423.899	357.543	3099.161
	.940	429.156	360.421	3215.516
	.950	435.152	363.685	3348.236
	.960	442.197	367.500	3504.186
	.970	450.857	372.164	3695.933
	.980	462.370	378.325	3950.865
	.990	480.515	387.967	4352.740